REMARKS

Upon entry of the present amendments, claims 1-14, 18-30, 32-35, 38-40, 184-188, 194 and 196-198 are pending.

Applicants have amended claim 1 to incorporate the limitations of claim 184, now canceled. Applicants have amended claim 23 to incorporate the limitations of claim 195, now canceled. Applicants have amended claim 32 to incorporate the limitations of claim 36 and 37, now canceled. Applicants have amended claims 187 and 198 to correct dependency. Applicants have amended claim 18 for clarification and have made grammatical amendments to claims 12, 18 and 39.

In the Response to Restriction Requirement of December 3, 2002, Applicants elected Group I in Paper No. 9. In response to the Examiner's request in the Office Action of February 27, 2003, page 2, Applicants now formally request that claims 31 and 41-183 be withdrawn from consideration.

Applicants have amended the specification to include a Statement Regarding Federally Sponsored Research or Development. Applicants have also amended the Abstract of the Disclosure to be a single paragraph on a separate sheet within the range of 50 to 150 words.

No new matter is added.

CLAIM OBJECTIONS

The Examiner has objected to several of the pending claims. Office Action of February 27, 2003, pages 3-4. In response, Applicants have amended claim 12 to correct the use of the verbs ("is" and "provides") before "a detectable signal". Applicants have also canceled claims 15-17 and 189-193. Applicants have amended claim 32 to delete the word "either" in line 5. Applicants have amended claim 38 to delete the second "is" in line 1. Finally, Applicants have amended claim 39 to delete the first "either" in line 3. Applicants have amended claim 198 to depend from claim 194. Accordingly, these claim objections are now moot.

CLAIM REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

The Examiner has rejected claims 18, 15-19, 187, and 189-193 for alleged indefiniteness. Applicants have canceled claims 15-17 and 189-193. Accordingly, these rejections are now moot.

The Examiner has rejected claim 18 for the recitation that the isolated tumor stem cell "further comprising a culture medium". Applicants have amended claim 18 to recite that "the solid tumor stem cell is situated in a culture medium". Accordingly, the metes and bounds of the claim are clear. This rejection is now moot.

The Examiner has rejected claim 187, which recites the limitation "the lineage markers" in line 1. The Examiner alleges that there is insufficient antecedent basis for this limitation in the claim. Applicants have amended claim 187 to depend from claim 4. Claim 187 now has sufficient antecedent basis for the term "lineage markers". This rejection is now moot.

Applicants respectfully request that these rejections under 35 U.S.C. § 112, second paragraph, be withdrawn.

CLAIM REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION

The Examiner has rejected claims 32-40, 188, and 198 as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse.

Applicants have amended claim 32 to recite a method for enriching a population of cells for solid tumor stem cells comprising contacting at least one reagent with a cell suspension derived from a solid tumor, wherein the reagent selectively binds to either a positive or a negative marker for tumor stem cells. Claim 32 as amended recites that the positive marker to which the reagent binds is CD44, B38.1 or ESA. The negative marker to which the reagent binds is CD2, CD3, CD10, CD14, CD16, CD31, CD45 CD64 or CD140b.

Applicants were in possession of the claimed invention as amended as of the filing date of the application. *See*, Office Action of March 27, 2003, page 5, referencing *Guidelines for Examination of Patent Applications under* 35 U.S.C. § 112, ¶ 1, *"Written Description" Requirement*; 66:4 Federal Register 3, II Methodology for Determining Adequacy of Written Description (January 5, 2001). The patent application describes an actual reduction to practice in which solid tumor stem cells are selected using positive or negative markers. *See, e.g.*, paragraph [230], "Cells were isolated with regard to CD44 expression." and TABLE 3. The invention was complete as evidenced by drawings, *e.g.*, FIG. 7, showing the tumorigenicity of the solid tumor

stem cells isolated by flow cytometry. The invention has been set forth in terms of distinguishing identifying characteristics, namely the recited cell markers by which the solid tumor stem cells can be selected.

Applicants respectfully request that this rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

CLAIM REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, ENABLEMENT, SCOPE

The Examiner has rejected claims 32-40, 188, and 198 for an alleged lack of enablement for enriching solid tumor stem cells using a combination of the genus of positive and negative markers. The Examiner alleges that the specification does not enable one to practice the invention commensurate in scope with these claims. Applicants respectfully traverse.

As stated in the Office Action of March 27, 2003, page 8, the factors to be considered when determining whether the disclosure satisfies the enablement requirements and whether undue experimentation would be required to make and use the claimed invention are summarized in the opinion of *In re Wands*, (858 F2d 731, 737, 8 USPQ 2d 1400, 1404, (Fed Cir. 1988)). These factors include but are not limited to the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, the breadth of the claims, and amount of direction provided. The factors most relevant to this rejection are the scope of the claims relative to the state of the art, and whether sufficient amount of direction or guidance are provided in the specification to enable one of skill in the art to practice the claimed invention without undue experimentation. "As concerns the breadth of a claim relevant to enablement, the only relevant concern should be whether the scope of enablement provided to one skilled in the art by the disclosure is commensurate with the scope of protection sought by the claims." M.P.E.P. § 2164.08, *citing In re Moore*, 439 F.2d 1232, 1236, 169 USPQ 236, 239 (CCPA 1971).

The specification enables one to practice the claimed invention. The claims as amended recite a method for enriching a population of cells for solid tumor stem cells comprising contacting at least one reagent with a cell suspension derived from a solid tumor, wherein the reagent selectively binds to either a positive or a negative marker for tumor stem cells. Claim 32 as amended recites that the positive marker to which the reagent binds is CD44, B38.1 or ESA.

The negative marker to which the reagent binds is CD2, CD3, CD10, CD14, CD16, CD31, CD45 CD64 or CD140b.

The skilled artisan intending to practice the invention would be able to identify a solid tumor stem cell using the methods provided in the specification. The skilled artisan could select solid tumor stem cells from other cancer cells using standard methods of flow cytometry (*see*, specification and references cited in paragraph [48]) and the cell markers provided in the specification. The skilled artisan could verify the tumorigenicity of the selected solid tumor stem cells using the *in vitro* tissue culture assays and the *in vivo* assays provided in the specification, especially the xenograft model provided in the specification at paragraphs [52] – [56] and EXAMPLE 3. The skilled artisan could also verify the selection of the solid tumor stem cells, *e.g.*, by testing the tumors produced in the xenograft model for the presence of solid tumor stem cells (*e.g.*, using a simple immunological test for the presence of cells with appropriate cell markers). See, EXAMPLE 10. The skilled artisan would be expected to use the xenograft model routinely, since the model provides a rapid and relatively economical way of maintaining a tumor bank from solid tumor stem cells (*see*, specification, paragraph [10]). No extensive experimentation would need to be carried out to identify optimal conditions for identifying and growing solid tumor stem cells further to what is provided in the specification.

Accordingly, the specification provides an enabling disclosure commensurate with the scope of the claims.

The Examiner also alleges that while claims 3, 4, 6, 26, 37, and 187 are drawn to a solid tumor stem cell lacking detectable levels of certain CD lineage markers (CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140) and a method of enriching the solid tumor stem cell by negative selection from solid tumor stem cells of any cell origin (lineage). The Examiner alleges that Janeway, Jr. *et al,. Immunobiology, The Immune System in Health and Disease,* 579-581, Appendix I (Current Biology Publications 1999) ("*Janeway, Jr.*") teaches that the lineage markers recited in these claims are for the hematopoietic lineage cells. The Examiner then concludes that the lineage markers would not normally be present in cells of other lineages, such as breast cancer cells. Applicants respectfully disagree with the Examiner's conclusion.

Janeway, Jr. shows that certain lineage markers are found on cells of the hematopoietic lineage. Because *Janeway Jr.*'s subject of interest is the immune system, the cells of which are

all from the hematopoietic lineage, this emphasis is appropriate. However, *Janeway, Jr.* does not state that lineage markers would not normally be present in cells of other lineages. Indeed, it is well known in the art that CD antigens are found on both hematopoietic and non-hematopoietic cells, including endothelial cells. *See, Immunology Link* website at <http://www.immunologylink.com/CDantigen.htm >. Note also that *Janeway, Jr.* discusses the marker CD44 on cells of the hematopoietic lineage, but CD44 is a positive stem cell marker.

In addition, one of skill in the art would not have assumed because certain markers are not present on at least some normal cells from a tissue means that the maker would necessarily not be present on tumor cells derived from that tissue. In addition, one of skill in the art would not have assumed that because a marker would necessarily not be present on some of the cells of a tumor, the marker must not present on some of the cells of a tumor. It was well known at the filing date of the application that solid tumors are composed of heterogeneous cell populations. *See*, specification, paragraphs [33] and [70]. The specification shows in many places that cells from a solid tumor expressing certain markers can be distinguished from cells from the same solid tumor not expressing the same marker, and *vice versa*.

Accordingly, the negative selection of solid tumor stem cells by reagents that bind to CD antigens is an appropriate basis for a dependent claim, not an inherent feature of solid tumor stem cells.

The Examiner also alleges that the specification fails to teach whether these lineage markers are selective for solid tumor stem cells but not non-tumorigenic cells of a solid tumor. Applicants respectfully traverse.

Applicants have amended the claims to recite that "the reagent that binds to a positive marker and/or that do not bind to the reagent that binds to a negative marker" Thus, it is not relevant whether the reagents that recognize lineage markers bind or not bind to non-tumorigenic cells of a solid tumor, so long as the reagents bind or not bind to the marker on the solid tumor stem cell, as recited in the claims. The specification teaches how to use reagents for positive and negative stem cell markers at paragraph [49].

> Solid tumor stem cell positive markers may also be present on cells other than solid tumor stem cells. Solid tumor stem cell negative markers may also be absent from cells other than solid tumor stem cells. While it is rare to identify a single marker that identifies a stem cell, it has often been possible to identify combinations of positive and negative markers that uniquely identify stem cells and allow their substantial enrichment in other contexts. Morrison *et al.*, *Cell* 96(5): 737-49 (1999); Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 92(22): 10302-6 (1995); Morrison & Weissman, *Immunity* 1(8): 661-73 (1994).

Applicants respectfully request that this rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

CLAIM REJECTIONS UNDER 35 U.S.C. § 102

The Examiner has rejected claims 1, 3-8, 15-18, 20, 21, and 189-193 as allegedly anticipated by Salmon *et al., New Eng. J. Med.* 298: 1321-7 (1978) ("*Salmon*"), as evidenced by *Janeway, Jr.* and Hartman *et al., Int. J. Cancer* 82: 256-67 (1999) ("*Hartman*"). Applicants respectfully traverse.

Anticipation is established by the express or inherent disclosure in a single prior art reference of every element of the claimed invention in question. M.P.E.P. § 2131. The claimed invention as amended is drawn to an isolated solid tumor stem cell derived from a solid tumor and is tumorigenic, wherein solid tumor stem cell expresses a marker selected from CD44 and epithelial specific antigen (ESA).

Salmon does not disclose all of the elements of the claimed invention. The claims as amended recite an isolated solid tumor stem cell that is derived from a solid tumor and is tumorigenic, where the solid tumor stem cell expresses a cell surface marker selected from CD44 and epithelial specific antigen (ESA). The Examiner alleges that *Salmon* discloses a "stem cell" (referencing *Salmon,* Table I, page 1323) derived from the ovarian carcinoma (malignant ovarian

epithelial cells), situated in a culture medium or affixed to 3% agar substrate (*Salmon,* page 1322) that gave rise to new tumor cell colonies and would form new tumors *in vivo* (*Salmon,* page 1321, 2nd paragraph). However, while *Salmon* does provide an *in vitro* bioassay system for cells derived from solid tumors, *Salmon* does not appear to show, either on page 1321, 2nd paragraph, or elsewhere in the publication, that the cells grown in the bioassay consistently form new tumors when transplanted *in vivo*. Also, *Salmon* does not disclose cells that express a cell surface marker selected from CD44 or epithelial specific antigen (ESA).

In summary, *Salmon* does not disclose all of the elements of the claimed invention. *Salmon* does not disclose an isolated cell that is tumorigenic. *Salmon* also does not disclose an isolated cell that expresses a cell surface marker selected from CD44 or epithelial specific antigen (ESA).

The Examiner also alleges that *Janeway, Jr.* teaches that the markers CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140 are expressed in blood cells or endothelial cells (Appendix I). The Examiner then concludes that the *Salmon* ovarian epithelial tumor cells would intrinsically lack detectable levels of expression of the recited CD markers. Applicants respectfully disagree with the Examiner's conclusion.

The Examiner has not shown that the *Salmon* ovarian epithelial tumor cells would intrinsically or inherently lack detectable levels of expression of the markers. "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic." M.P.E.P. § 2112 (emphasis in original), *citing In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because determination of inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art). "In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original), *cited in* M.P.E.P. § 2112. Here, the Examiner appears to have assumed that the lack of detection of certain CD markers on some cells that are members of the class of epithelial cells means that these CD markers would necessarily not be found on solid

tumor stem cells derived from epithelial tumors. The Examiner has shown no evidence to support this apparent assumption.

One of skill in the art would not have assumed because certain markers are not present on at least some normal cells from a tissue means that the maker would necessarily not be present on tumor cells derived from that tissue. In addition, one of skill in the art would not have assumed that because a marker would necessarily not be present on some of the cells of a tumor, the marker must not present on some of the cells of a tumor. It was well known at the filing date of the application that solid tumors are composed of heterogeneous cell populations. *See*, specification, paragraphs [33] and [70]. The specification shows in many places that cells from a solid tumor expressing certain markers can be distinguished from cells from the same solid tumor not expressing the same marker, and *vice versa*.

In summary, the Examiner has not shown that the *Salmon* ovarian epithelial tumor cells would intrinsically or inherently lack detectable levels of expression of the recited CD markers.

The Examiner also alleges that *Hartman* teaches that ovarian epithelial cancer cells (carcinoma) express an "epithelial specific antigen" (1st paragraph, page 256). The Examiner concludes that the *Salmon* ovarian epithelial tumor cells would intrinsically express ESA. Applicants respectfully disagree with the Examiner's conclusion.

The term "ESA" is well known to those of skill in the art to refer to a specific antigen, not a general group of antigens that are found on epithelial cells. Antibodies that bind to ESA, such as the ones used in the method of the invention, are commercially available from several sources and were available at the filing date of the patent application. An antibody that recognizes epithelial specific antigen (ESA) binds to a 40 kDa transmembrane epithelial glycoprotein (EGP40), also identified as epithelial cellular adhesion molecule (Ep-CAM). The gene for ESA has been fully sequenced. ESA has been detected on many but not all cancer cells. By contrast, *Hartman* shows that the presence of MUC1 (mucin 1) on cancer cells. MUC1 is a different and immunologically distinct protein from ESA. Furthermore, *Hartman* does not show that cancer cells that do or do not express MUC1 differ in their ability to form tumors.

In summary, *Hartman* does not show that the *Salmon* ovarian epithelial tumor cells would intrinsically express ESA.

Applicants respectfully request that the rejection of claims 1, 3-8, 15-18, 20, 21, and 189-193 as anticipated by *Salmon* be withdrawn.

The Examiner has rejected claims 1, 3-8, 15-18, 20, 22, and 189-193 as allegedly anticipated by U.S. Pat. No. 4,411,990 to Salmon *et al.* ("*Salmon '990*"). Applicants respectfully traverse.

The Examiner alleges that *Salmon '990* discloses tumor stem cells derived from various solid tumors, including ovarian and lung carcinomas (*Salmon '990,* Table 1). The Examiner alleges that *Salmon '990* also teaches tumor cells that are tumorigenic and would form new tumors *in vivo* (*Salmon '990,* col. 1, line 23).

For the reasons discussed above regarding the *Salmon* scientific publication, *Salmon '990* does not disclose all of the elements of the claimed invention. *Salmon '990* does not disclose an isolated cell that is tumorigenic. *Salmon '990* does not appear to show, either at col. 1, line 23, or elsewhere in the patent, that the cells grown in the bioassay form new tumors when transplanted *in vivo*. *Salmon '990,* col. 1, line 23 states only that the "colony forming assays, either in vitro or in vivo, can be used to study" the cells. *Salmon '990* does not disclose cells that express a cell surface marker selected from CD44 or epithelial specific antigen (ESA).

Because *Salmon '990* does not disclose all of the elements of the claims, *Salmon '990* does not anticipate the claimed invention.

The Examiner also alleges that these *Salmon '990* cells are epithelial tumors. The Examiner then concludes that the *Salmon '990* cells would intrinsically express ESA and lack the expression of CD2, CD3, CID10, CD14, CD16, CD31, CD45, CD64, and CD140. Applicants respectfully disagree with the Examiner's conclusion.

As discussed above regarding the *Salmon* scientific publication, the Examiner has not shown that the *Salmon* ovarian epithelial tumor cells would intrinsically or inherently lack detectable levels of expression of the markers. The Examiner appears to have assumed that the lack of detection of certain CD markers on some cells that are members of the class of epithelial cells means that these CD markers would necessarily not be found on solid tumor stem cells derived from epithelial tumors. The Examiner has shown no evidence to support this apparent assumption. Applicants submit that one of skill in the art would not have assumed that because a

marker would necessarily not be present on some of the cells of a tumor, the marker must not present on some of the cells of a tumor.

In addition, the Examiner has not shown that epithelial cancer cells express ESA. *Hartman* shows that the presence of MUC1 (mucin 1) on epithelial cancer cells. MUC1 is a different and immunologically distinct protein from ESA.

Accordingly, *Salmon '990* and *Hartman* do not show that solid tumor stem cells express ESA and fail to express the recited CD markers.

Applicants respectfully request that the rejection of claims 1, 3-8, 15-18, 20, 22, and 189-193 as anticipated by *Salmon '990* be withdrawn.

The Examiner has rejected claims 1-8, 15-18, 23-29, 184-187, and 189-197 as allegedly anticipated by Martin *et al., Exp. Hematol.* 26:252-64 (1998) ("*Martin*"), as evidenced by U.S. Pat. No. 4,612,282 to Schlom *et al.* ("*Schlom 282*"). Applicants respectfully traverse.

The Examiner alleges that *Martin* teaches an enriched population of metastatic tumor cells derived from breast cancer epithelial cells, wherein the tumor cells were situated in a culture medium after collection, and were 25-fold enriched than the original collected tumor cells (*see, Martin*, Abstract), wherein the cell population were negative for CD45 (*Martin*, page 253, right column), wherein about 4% of the cells in the enriched population express CD44v6 (*Martin*, page 257). The Examiner alleges that *Martin* goes on to teach that such a result is consistent with the previous study in animal models "showing that only a small percentage of the circulating tumor cells is able to successfully initiate metastatic colonies" (*Martin*, page 262, last paragraph).

Martin does not disclose all of the elements of the claimed invention. *Martin* began with breast cancer and prostate cancer cells from a tumor bank. *Martin*, page 253, col. 1, 5th paragraph. These tumor cells would have been heterogeneous, not enriched for solid tumor stem cells. *Martin* also used blood samples from carcinoma patients. *Martin*, page 253, col. 1, 6th paragraph. There is no showing that these cells seeded into the blood from carcinomas would have been enriched for solid tumor stem cells. *Martin* did not use flow cytometry to enrich for solid tumor stem cells from the population of heterogeneous cancer cells. Rather, *Martin* used flow cytometry to "detect and enumerate carcinoma cells" (*Martin*, page 253, col. 2, 6th

paragraph) that had been seeded into the blood. The "enrichment" disclosed by Martin is and enrichment of heterogeneous cancer cells from normal non-cancerous blood cells, not the enrichment of solid tumor stem cells from other cells of the tumor. *See, Martin,* page 254, col. 2., "Enrichment of tumor cells from buffy coat cells spiked with BT474 breast cancer cells" and page 255, col. 1, "Enrichment of tumor cells from blood of carcinoma patients".

Martin does not disclose the enrichment of solid tumor stem cells that express a cell surface marker selected from CD44 or epithelial specific antigen (ESA) from other cells of the tumor. The Examiner cites *Martin*, page 257, col. 2, which states that "In patient PC4 approximately 4% of the cytokeratin 8^+ cells expressed CD44v6 [an exon-v6-containing splice variant of CD44]. Normal leukocytes in this patient were all negative for CD44v6 staining". *Martin* does not show that this represents an enrichment for solid tumor stem cells, rather than a normal 4% expression of CD44v6 by unenriched tumor cells. *Martin* also does not show that an enriched population of solid tumor stem cells is enriched for CD44 expression. All *Martin* shows is that in one patient out of 34 tested (*see, Martin*, page 253, col. 1, 6^{th} paragraph), some of the cells tested by immunoflurorescence microscopy express an aberrant splice variant of CD44. *See, Martin*, page 262, last paragraph.

Martin does not disclose an isolated cell that is tumorigenic. *Martin* does not appear to show, either in page 262, last paragraph, or elsewhere in the publication, that the cells obtained from the blood form new tumors when transplanted *in vivo*. The full paragraph cited by the Examiner (*Martin,* page 262, last paragraph) does not show that the cells are tumorigenic. The full paragraph states that:

> A mAb directed against CD44v6 was used for counterstaining of the MACS-enriched fraction. It has been shown that CD44 variant glycoproteins containing sequences encoded by exon 6 confer full metastatic potential on carcinoma and sarcoma cell lines [36, 37]. A number of analyses of human tumor material have also indicated a potential involvement of CD44 variant isoforms in human tumor dissemination [38,39]. We found CD44v6 coexpression in a small percentage (4%) of all cytokeratin 8+ cells isolated from a blood sample of a prostrate cancer patient. Whether these cells represent more aggressive tumor cells and the CD44v6 negative cells remain quiet remains to be seen. Such a hypothesis would be in accordance with earlier animal models showing that only a small percentage of the circulating tumor cells is able to successfully initiate metastatic colonies [40-42].

(Emphasis added). *Martin* does not say that the carcinoma cells isolated from blood are among the small percentage of cells that are metastatic (not tumorigenic, as recited in the claims). Martin does not state definitively that the CD44v6 splice variant is indicative of solid tumor stem cells has nothing to say regarding the CD44 generally, without any RNA splice variation. The *Martin* "counterstaining" does not show that $CD44v6^+$ positive cells are enriched relative to other cells from the same tumor.

Because *Martin* does not disclose all of the elements of the claims, *Martin* does not anticipate the claimed invention.

The Examiner alleges that *Martin* breast epithelial tumor cells express ESA (cytokeratin-8), and lack detectable levels of expression of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

Regarding the ESA marker, cytokeratin-8 is not the protein that is the ESA marker. *See*, discussion above regarding *Salmon, Hartman* and the ESA marker.

Regarding the CD markers, claim 32 as amended does not recite a negative selection by a reagent that binds to the CD45 marker. Moreover, *Martin* does not distinguish solid tumor stem cells from non-tumorigenic cancer cells. *Martin* shows the selection of carcinoma cells based upon the lack of the marker CD45. CD45 is present on leukocytes. *Martin*'s negative selection for $CD45^+$ cells removes leukocytes, but does not enrich solid tumor stem cells that fail to express the cell surface marker CD45 from other cells of the tumor.

Accordingly, *Martin* does not show that solid tumor stem cells express ESA and fail to express the recited CD markers.

The Examiner also alleges that the *Martin* cells taught would intrinsically express B38.1. The Examiner alleges that *Schlom '282* teaches that B38.1 intensely expressed in mammary carcinoma (*Schlom '282*, TABLE 1), possess a "pancarcinoma" pattern of binding activity (*Schlom '282*, col. 7, lines 2-4), and could be used for diagnosis of primary and metastatic breast tumor (*Schlom '282*, col. 10, lines 1-51). Applicants respectfully traverse the Examiner's allegation that these marker limitations are inherently present in the *Martin* cells.

Schlom '282 does not disclose that the B38.1 marker can be used to select solid tumor stem cells from other cancer cells of a solid tumor. *Schlom '282,* col. 6, line 66 to col. 7, line 4, discloses that:

> B38.1 bound to BT-20, MCF-7, ZR-75-1, A549 lung carcinoma cell line, A 431 vulva epidermoid carcinoma line and KB oral epidermoid carcinoma line (not to carcinoma and metanoma cell lines tested). Antibody B38.1 thus appears to possess a "pancarcinoma" pattern of binding activity.

The cancer cells described by *Schlom '282* contain heterogeneous cancer cells. *Schlom '282* does select solid tumor stem cells from other cancer cells of a solid tumor. The "pancarinoma" nature described by *Schlom '282* refers to the ability of the B38.1 antibody to bind to cells from several different cancers, not to an ability of the antibody to bind to all cells of a cancer. The fact that *Schlom '282* found that B38.1 did not bind to all cancers could be caused by any of a number of possible factors.

Accordingly, *Martin* and *Schlom '282* do not show that solid tumor stem cells express the B38.1 marker.

The Examiner also alleges that the recitation of "wherein the solid tumor stem cell expresses lower levels of the marker CD24" in claims 186 and 197 describes an inherent property of a tumor stem cell. The Examiner alleges that *Martin* disclose the same type of solid tumor stem cells as disclosed in the present specification, *i.e.* derived from breast epithelial tumor, expressing CD44 marker and ESA, negative in CD45 marker, and were tumorigenic (metastasis), therefore, these cells would also express lower levels of the marker CD24 than mean expression of CD24 by nontumorigenic cancer cells. Applicants respectfully traverse the Examiner's allegation that these limitations are inherently present in *Martin* cells.

First, *Martin* does not disclose the same type of solid tumor stem cells as disclosed in the present specification, as shown above. Second, the Examiner has not provided any evidence outside of the Applicants' own disclosure in the specification that the expression of lower levels of the marker CD24 describes an inherent property of a solid tumor stem cell. The Examiner has not provided any reference that indicates that expression of lower levels of the marker CD24 is necessarily a property of a solid tumor stem cell.

Accordingly, the expression of lower levels of the marker CD24 is an appropriate basis for a dependent claim, not an inherent feature of solid tumor stem cells.

Applicants respectfully request that the rejection of claims 1-8, 15-18, 23-29, 184-187, and 189-197 as anticipated by *Martin* be withdrawn.

The Examiner has rejected claims 1, 3-7, 9-13, 15-18, 184, and 189-193 as allegedly anticipated by Nierodzik *et al., Blood* 92: 3694-3700 (1998) ("*Nierodzik*"). Applicants respectfully traverse.

The Examiner alleges that *Nierodzik* teaches an isolated population of tumor cells derived from colon carcinoma and melanoma and having a pulmonary metastatic phenotype (*Nierodzik,* Abstract). According to the Examiner, the *Nierodzik* cells are solid tumor stem cells, because they are allegedly tumorigenic.

Nierodzik does not disclose all of the elements of the claimed invention. *Nierodzik* does not disclose cells that express a cell surface marker selected from CD44 or epithelial specific antigen (ESA). *Nierodzik* does not disclose a cell that is a necessarily tumorigenic. *Nierodzik* only discloses that the addition of thrombin, along with other factors can "induce a metastatic phenotype". *Nierodzik*, page 3698, col. 2.

Because *Nierodzik* does not disclose all of the elements of the claims, *Nierodzik* does not anticipate the claimed invention.

The Examiner also alleges that the *Nierodzik* colon epithelial tumor cells intrinsically express ESA and lack detectable levels of expression of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b. Applicants respectfully traverse the Examiner's allegation that these limitations are inherently present in *Nierodzik* cells.

First, *Nierodzik* does not disclose a solid tumor stem cell as disclosed in the present specification, as shown above. Second, the Examiner has not provided any evidence outside of the Applicants' own disclosure in the specification that the expression of ESA or the lack of detectable expression of certain CD markers describes an inherent property of a solid tumor stem cell. The Examiner has not provided any additional reference that indicates that the expression of ESA or the lack of detectable expression of certain CD markers is necessarily a property of a solid tumor stem cell.

Accordingly, the expression of ESA or the lack of detectable expression of certain CD markers is an appropriate basis for a dependent claim, not an inherent feature of solid tumor stem cells.

Applicants respectfully request that the rejection of claims 1, 3-7, 9-13, 15-18, 184, and 189-193 as anticipated by *Nierodzik* be withdrawn.

The Examiner has rejected claims 1, 3, 4, 6, 9-18, and 189-193 as allegedly anticipated by Bromberg *et al., Proc. Natl. Acad. Sci. USA* 92: 8205-9 (1995) ("*Bromberg*"). Applicants respectfully traverse.

The Examiner alleges that *Bromberg* teaches an isolated tumor cell derived from melanoma, whose tumorigenic ability was tested by introducing the transfected cells in SCID mice (*Bromberg,* Abstract).

Bromberg does not disclose all of the elements of the claimed invention. *Bromberg* does not disclose cells that express a cell surface marker selected from CD44 or epithelial specific antigen (ESA). *Bromberg* does not disclose a cell that is necessarily tumorigenic. Instead, *Bromberg* discloses that the addition of thrombin, along with other factors can "induce a metastatic phenotype". *Bromberg*, page 3698, col. 2.

Because *Bromberg* does not disclose all of the elements of the claims, *Bromberg* does not anticipate the claimed invention.

The Examiner alleges that the *Bromberg* melanoma cells would not express the CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b markers. Applicants respectfully disagree. Applicants respectfully traverse the Examiner's allegation that these limitations are inherently present in *Bromberg* cells.

First, *Bromberg* does not disclose a solid tumor stem cell as disclosed in the present specification, as shown above. Second, as discussed above regarding *Nierodzik*, the Examiner has not provided any evidence outside of the Applicants' own disclosure in the specification that the lack of detectable expression of certain CD markers describes an inherent property of a solid tumor stem cell. The Examiner has not provided any additional reference that indicates that the expression of ESA or the lack of detectable expression of certain CD markers is necessarily a property of a solid tumor stem cell.

Applicants respectfully request that the rejection of claims 1, 3, 4, 6, 9-18, and 189-193 as anticipated by *Bromberg* be withdrawn. Applicants respectfully request that all of these rejections under 35 U.S.C. § 102 be withdrawn.

CLAIM REJECTIONS UNDER 35 U.S.C. § 103

The Examiner has rejected claims 23, 30, 32-40, 188, and 198 as allegedly unpatentable over *Martin* in view of *Salmon '990.* The Examiner alleges that it would have been obvious to combine the method steps taught by *Martin* and *Salmon '990* for enriching breast cancer stem cells with a reasonable expectation of success. The Examiner also alleges that the ordinary skilled artisan would have been motivated to modify the claimed invention because if the tumor sample is from a solid tumor, the cells have to be dissociated before they could contact an antibody for enrichment. Applicants respectfully traverse.

To establish a *prima facie* case of obviousness, "the prior art reference (or references when combined) must teach or suggest all the claim limitations." M.P.E.P. § 2143. Applicants have amended claims 32-40, 188, and 198 to recite a method for enriching a population of cells for solid tumor stem cells comprising (a) dissociating a solid tumor to form a cell suspension, (b) contacting the dissociated cells with at least one reagent that selectively binds to a positive marker for a solid tumor stem cell, or a combination of reagents that selectively bind either a positive or a negative marker for a solid tumor stem cell, wherein the solid tumor stem cell positive marker is a marker CD44, B38.1 or ESA and the negative marker is CD2, CD3, CD10, CD14, CD16, CD31, CD45 CD64 and CD140b, (c) selecting cells that bind to the positive marker/or do not bind to the negative marker, and (d) isolating the selected solid tumor stem cell; wherein the solid tumor is a sarcoma or epithelial cancer, preferably a breast cancer, wherein the selection is performed by flow cytometry, FACS, and/or magnetic selection, wherein the reagent is an antibody, wherein the reagent is conjugated to a fluorochrome or to magnetic particles, wherein the positive marker is an epithelial specific antigen.

The combination of *Martin* and *Salmon '990* does not contain all the limitations of the claims as amended. The combination of *Martin* and *Salmon '990* does not teach an enriched population of solid tumor stem cells where the cells are tumorigenic. *Salmon '990* does not appear to show, either at col. 1, line 23, or elsewhere in the patent, that the cells grown in the

bioassay form new tumors when transplanted *in vivo*. *Salmon '990,* col. 1, line 23 states only that the "colony forming assays, either in vitro or in vivo, can be used to study" the cells. *Martin* does not appear to show, either in page 262, last paragraph, or elsewhere in the publication, that the cells obtained from the blood form new tumors when transplanted *in vivo*.

The combination of *Martin* and *Salmon '990* also does not teach an enriched population of cells that express a cell surface marker selected from CD44 or epithelial specific antigen (ESA). The combination does not teach a method of enriching a heterogeneous population of cancer cells for a solid tumor stem cells. Furthermore, as discussed above, the *Martin* cells are heterogeneous cancer cells enriched from normal non-cancerous blood cells, not the enrichment of solid tumor stem cells from other cancer cells of the solid tumor.

Because the combination of *Martin* and *Salmon '990* does not teach or suggest all the claim limitations of the claims, the combination does not render obvious the claimed invention.

Claim 30 is drawn to a tumor cell population that is at least 50-fold enriched. The Examiner admits that *Martin* teaches a tumor cell population that is 25-fold enriched, not 50-fold enriched. However, the Examiner alleges that 50-fold enrichment could be achieved by repeating the steps (b) through (d). Applicants respectfully disagree.

The *Martin* method does not result in a 25-fold enrichment of solid tumor stem cells. *Martin* teaches the enrichment of heterogeneous cancer cells from normal non-cancerous blood cells, not the enrichment of solid tumor stem cells from other cancer cells of the solid tumor. Evan an unlimited repetition of steps (b) through (d), as suggested by the Examiner, would not result in an enriched population of solid tumor stem cells and so would not result in the claimed invention.

The Examiner has rejected claims 1, 18, and 19 as allegedly unpatentable over *Salmon '990* in view of Jeffries *et al., Mol. Cell Biol.* 20: 3928-41 (June 2000) ("*Jeffries*"). The Examiner alleges that it would have been obvious to modify the method taught by *Salmon '990* by simply adding Notch ligand to the culture medium for culturing tumor stem cells with a reasonable expectation of success. Applicants respectfully traverse.

The Examiner admits that *Salmon '990* disclose adding nutrients such as METGF to culture system to promote stem cell colony growth (column 4, lines 35-60) but fails to teach

adding a Notch ligand in the culture system. The Examiner alleges that *Jeffries* teaches that interaction between Notch proteins and their proposed ligands initiate a signaling cascade that governs cell fate decisions (*Jeffries*, page 3928, 1st paragraph), that such interaction has a direct role in the transformation of cells and can cooperate with cellular proto-oncogenes to accelerate tumorigenesis in different tissue types (*Jeffries*, page 3928, 3rd and 4th paragraph).

The Examiner's allegation is based on improper hindsight reasoning. The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention. M.P.E.P. § 2141, *citing Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986). Here, however, the rejection does not take into account only knowledge that was within the level of ordinary skill in the art at the time the claimed invention was made, but rather includes knowledge gleaned only from applicant's disclosure. *See*, M.P.E.P. § 2145, *citing In re McLaughlin* 443 F.2d 1392, 1395, 170 USPQ 209, 212 (CCPA 1971). Before the Applicants' disclosure in the specification, it was not known that solid tumors contain solid tumor stem cells. Thus, one of skill in the art would not have been motivated to add Notch ligand to a culture of solid tumor cells. The reason for adding a Notch ligand to the culture medium is to enhance the self-renewal of solid tumor stem cells in the culture medium. For example, the specification, paragraph [58], states that "In *C. elegans,* Notch is required for germ line stem cell self-renewal. Berry *et al.*, *Development* 124(4): 925-36 (1997)." *See also*, specification, paragraphs [240] and [306].

In addition, the combination of *Salmon '990* and *Jeffries* does not teach all of the limitations of the claims. As discussed above, *Salmon '990* does not teach the solid tumor stem cells of the invention. *Jeffries* makes no mention of solid tumor stem cells.

Accordingly the combination of *Salmon '990* and *Jeffries* does not render obvious the claimed invention. Applicants respectfully request that both these rejections under 35 U.S.C. § 103 be withdrawn.

CONCLUSION

If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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